Chapter 3

Approaches to optimising dietary protein to energy ratio in African catfish, *Clarias gariepinus* (Burchell, 1822)

3.1 INTRODUCTION

The dietary protein to energy ratio in fish diets is of great importance. Levels of dietary protein and energy not only influence growth and body composition, but also digestive enzyme activities and plasma metabolites in various fishes (Wilson *et al*., 1985; Lovell, 1989; Shimeno *et al*., 1995a,b; Gangadhara *et al*., 1997; McGoogan and Gatlin III, 2000; Yamamoto *et al*., 2000).

Protein utilisation depends on a great number of factors (Steffens, 1981) and increasing the protein level of the diet does not result in better protein utilisation. Protein affects energy utilisation therefore replacing protein with lipid or carbohydrate might create a protein-sparing effect. Providing adequate energy through dietary lipids can minimise the use of more costly protein as an energy source (De Silva *et al*., 1991; Jayaram and Beamish, 1992; Bazaz and Keshavanath 1993; Van der Meer *et al*., 1997; Jantrarotai *et al*., 1998; Company *et al*., 1999; McGoogan and Gatlin III, 2000). High-energy diets can also lead to excessive deposition of carcass lipids (Page and Andrews, 1973; Watanabe, 1982) and reduced growth rate (Daniels and Robinson, 1986). Excess carcass lipid accumulation and reduced growth rate due to increased dietary energy have also been shown for African catfish, *Clarias gariepinus* (Machiels and Henken, 1985), channel catfish (Mohsen and Lovell, 1990) and Indian major carp (Hassan *et al*., 1995; Hassan and Jafri, 1996).

To reduce feeding costs in aquaculture, approaches to reducing dietary protein levels or improving protein utilisation have been studied extensively. Most studies concentrate on increasing dietary energy levels, or lowering the P/E ratio, to reduce the amount of protein in fish diets and have been confined mainly to studies of growth performance such as in salmon,

trout, channel catfish, red drum, tilapia, hybrid *Clarias* catfish, snakehead and carp (Page and Andrews, 1973; Garling and Wilson 1976; Takeuchi *et al*., 1978; Reis *et al*., 1989; El-Sayed and Teshima, 1992; Hassan *et al*., 1995; Hassan and Jafri, 1996; Samantaray and Mohanty, 1997; Jantrarotai *et al.,* 1998; Thoman *et al*., 1999).

Information on nutrient requirements and appropriate P/E ratios in African catfish, *Clarias gariepinu*s fed practical fish meal based diets is restricted. Previous studies on optimum protein to energy ratio for *C. gariepinus* adult and sub-adult fish 120-233g (Pantazis, 1999), 40g (Henken *et al.,* 1986), 40-120g (Machiels and Henken, 1985) used semi-purified and purified diets. In semi-intensive and intensive production systems there has been a trend towards higher yields with young immature fish (8-12g) capable of rapid and efficient growth. In addition, the purpose of fish culture is to increase the weight of fish in the shortest possible time under economically acceptable conditions (Steffens, 1989). Therefore, it was felt advantageous to establish an optimum protein to energy ratio in the current study using practical fishmeal based diets 10-13g juvenile.

In general, dietary protein requirements seem to be of the order of 40% for *Clarias gariepinus* and hybrid *Clarias* catfish and somewhat lower for *Clarias batrachus* (Machiels and Henken, 1985; Degani *et al.,* 1989; Uys 1989; Khan and Jafri, 1990; Singh and Singh, 1992; Jantrarotai *et al*., 1996, 1998). Unfortunately, dietary energy requirements are not uniformly expressed (GE, DE or ME), hampering objective comparison between studies. Machiels and Henken (1985) reported, for *Clarias gariepinus*, that growth performance and protein utilisation were better at 23 kJ/g GE, at a crude protein level of over 40%, resulting in P/E ratios between 16

mg and 26.4 mg protein kJ^{-1} of GE. Growth performance and protein utilisation were better at 11.52 kJ. g^{-1} of DE, at a crude protein level of 40%, resulting in P/E ratio 34.72 mg protein kJ^{-1} of DE in hybrid *Clarias* catfish (Jantrarotai *et al*., 1998). Moreover, optimum P/E ratios depend on temperature in *Clarias gariepinus* (Henken *et al*., 1986).

The objective of the present study was to investigate dietary protein and energy interaction and its influence on the growth, feed and protein utilisation and body composition leading to optimisation of protein to energy ratios (P/E ratio) for African catfish, *Clarias gariepinus*. To further understand the metabolic effects of the dietary nutrients, variations in digestive enzyme activities, plasma metabolites and histological examination of liver and intestine tissue, were also considered.

3.2 MATERIALS AND METHODS

3.2.1 Experimental System

The experimental system described in Section 2.1 and Figure 2.1 was used to conduct an experiment to optimise the dietary protein to energy ratio for African catfish, *Clarias gariepinus* (Burchell, 1822).

3.2.2 Experimental Fish

African catfish, *Clarias gariepinus* were used as the species in this study. Ten-week (10.89 ± 0.04 g) fingerlings were obtained from broodstock maintained at the Institute of Aquaculture, University of Stirling following the procedure detailed in Section 2.2. Fish were randomly assigned into groups of 20 fish and each group was placed in an individual 30-L cylindrical tank as described in Section 2.1 and as shown in Figure 2.1.

3.2.3 Experimental Diets

Six experimental diets were formulated to contain two levels of protein (33 and 43%), each with three levels of lipid (4, 8 and 12%), in order to produce a range of protein to energy ratios. Protein to energy ratios ranged from 15.49 to 21.28 mg protein per kJ of GE. Diets are referred to by two numbers separated by a '/', the first number being the dietary percentage protein the second number the percentage lipid. Composition of the experimental diets and their proximate analyses are shown in Table 3.1. Diet formulation and preparation were as described in Sections 2.3.1 and 2.3.2. All diets were adequate in essential amino acids (EAA) on a percentage-of-protein basis. The essential amino acid (EAA) composition of diet 3 and the requirements for EAA in this species are shown in Table 3.2.

3.2.4 Experimental Practices

Fish were acclimated to the experimental system using a commercial trout diet (Trout Fry 02 crumble 1.00 ɒ 1.50 mm, BioMar Ltd., Scotland) for two weeks before the start of the experiment. Acclimation and periodical weighing were as described in Section 2.4.1. Fish were offered 5% of their body weight per day sub-divided into three equal feeds at 10:00, 14:00 and 18:00 h daily. Food was offered taking care to provide small amounts of food at a time to be sure that the fish ate all of the diet offered. The experiment was conducted for 8 weeks with three replicates per treatment. Faeces collection was as described in Section 2.4.4.

Before commencement of the feeding trial, 10-12 fish were randomly sacrificed with an overdose of benzocaine solution, and triplicate pooled samples were taken for determination of initial whole body composition. At the end of the experiment, all fish were weighed and counted, and 12 fish from each tank were collected for determination of whole body composition, histopathology, organ indices, liver lipid, liver glycogen, digestive enzymes and blood plasma components.

3.2.5 Water Quality Management

Water quality management was as described in Section 2.5. All values were within the optimum range for this species (Table 2.4, Section 2.1).

Table 3.1 Formulation and composition of the experimental diets and proximate analysis (Percentage dry weight)

	Diets number (Designation : Protein / Lipid, %)					
Diet no.:	1	2	3	4	5	6
(Protein / Lipid), (%)	(33/4)	(33/8)	(33/12)	(43/4)	(43/8)	(43/12)
Ingredients (g / 100g):						
Fishmeal (Herring type)[1]	16.00	16.00	16.00	22.00	22.00	22.00
Soybean meal (Dehulled, solvent extract)[2]	24.00	24.00	24.00	33.00	33.00	33.00
Wheat flour (Whole wheat)[3]	30.00	30.00	30.00	30.00	30.00	30.00
Fish oil	1.05	3.05	5.05	0.75	2.75	4.75
Corn oil	1.05	3.05	5.05	0.75	2.75	4.75
Vitamin premix[4]	1.00	1.00	1.00	1.00	1.00	1.00
Mineral premix[5]	1.00	1.00	1.00	1.00	1.00	1.00
Chromic oxide (Cr_2O_3)	0.50	0.50	0.50	0.50	0.50	0.50
Carboxymethyl cellulose (Binder)[6]	2.00	2.00	2.00	2.00	2.00	2.00
Corn starch	15.40	15.40	15.40	0.00	0.00	0.00
α-Cellulose	8.00	4.00	0.00	9.00	5.00	1.00
Proximate analysis:						
% as fed:						
Moisture	12.71	10.51	11.09	13.34	13.80	10.05
% Dry wt. basis:						
Crude Protein	33.25	33.42	33.12	43.79	43.51	43.29
Crude Fat	4.35	7.81	10.88	4.28	8.23	12.08
Ash	5.83	5.82	5.74	7.11	7.05	7.07
Fibre	7.43	4.87	2.12	8.33	5.95	3.15
NFE[7]	49.14	48.08	48.14	36.49	35.26	34.41
Cr_2O_3	0.51	0.47	0.45	0.53	0.47	0.43
GE (kJ/g)[8]	19.46	20.53	21.38	20.58	21.18	22.14
P / GE ratio[9]	17.09	16.28	15.49	21.28	20.54	19.55
DE (kJ / g)[10]	11.48	12.57	13.62	11.69	12.86	14.04
P / DE ratio[11]	28.75	26.25	24.23	36.78	33.43	30.63

Proximate analysis (% dry wt.):

1. Moisture: 7.59; Crude protein: 72.43; Crude fat: 9.85; Fibre: 0.54; Ash: 10.52.
2. Moisture: 8.38; Crude protein: 62.59; Crude fat: 1.86; Fibre: 1.68; Ash: 6.67.
3. Moisture: 11.25; Crude protein: 11.53; Crude fat: 1.14; Fibre: 2.69; Ash: 1.73.
4. As listed in Table 2.1, Section 2.3.1
5. As listed in Table 2.2, Section 2.3.1
6. Carboxymethyl cellulose – Sodium salt, high viscosity
7. NFE = Nitrogen free extractives, calculated as 100 – (% protein + % Lipid + % Ash + % Fibre)
8. GE = Gross energy content
9. P / GE ratio = Protein to energy ratio in mg protein / kJ of GE
10. DE = Digestible energy content
11. P / DE ratio = Protein to energy ratio in mg protein / kJ of DE

Table 3.2 Essential amino acid compositions (EAA, g/100g protein) of experimental diet 3, and EAA requirements of African catfish, *Clarias gariepinus*

Amino acids:	Diet 3	EAA requirements [a]
Arginine	4.76	4.30
Histidine	1.92	1.50
Isoleucine	2.97	2.60
Leucine	5.66	3.50
Lysine	4.52	5.00
Methionine[b]	0.67	2.30
Phenylalanine[c]	3.24	5.00
Threonine	3.19	2.00
Valine	3.59	3.00

[a] Requirement of a related species, channel catfish (NRC, 1993)

[b] In the absence of dietary cystine (NRC, 1993)

[c] Dietary protein contained 1.49 percent tyrosine. With 0.6 percent tyrosine the phenylalanine requirement was 2.0 percent of the dietary protein (NRC, 1993)

3.2.6 Experimental Analyses

3.2.6.1 Proximate Analyses

Proximate analysis (moisture, crude protein, crude lipid and ash) of carcasses, feed ingredients and experimental diets were determined as described in Sections 2.6.1.1, 2.6.1.2, 2.6.1.3 and 2.6.1.5. Crude fibre and gross energy contents of experimental diets were determined as described in Sections 2.6.1.4 and 2.6.1.7.1, while chromic oxide levels in fish faeces and experimental diets were determined as described in Section 2.6.1.8. Fish within each group were pooled for carcass analysis. Final values for each group represent the arithmetic mean of three replicates, all samples were analysed in triplicate.

3.2.6.2 Growth and Feed Performance

Growth and feed performance, nutrient digestibility determination and somatic indices were calculated according to the methods described in Sections 2.6.2.1.1, 2.6.2.1.2, 2.6.2.1.3, 2.6.2.1.4, 2.6.2.2, 2.6.2.3, 2.6.2.4 and 2.6.2.5.

3.2.6.3 Liver Lipid and Glycogen Determination

Liver lipid and liver glycogen were estimated as described in Section 2.7 and 2.8.

3.2.6.4 Digestive Enzyme Assays

Digestive enzyme (protease and lipase) assays were as described in Section 2.10.

3.2.6.5 Blood Plasma Assays

Plasma assays were performed as described in Section 2.11.3.

3.2.6.6 Amino acid Analysis

Amino acid analyses were as described in Section 2.12.

3.2.6.7 Histological Analysis

Histological analyses of fish livers and intestines were performed as described in Section 2.9.

3.2.6.8 Statistical Analysis

Statistical analyses were carried out as described in Section 2.13.

3.3 RESULTS

3.3.1 Growth and Feed Performance

No mortality nor external clinical symptoms occurred in any treatment during the period of this study. Growth responses are shown in Table 3.3 and graphically in Figure 3.1. Growth response was significantly ($P < 0.05$) affected by the inclusion level of dietary protein and energy. Weight gain increased in response to higher dietary protein, but the highest dietary energy level resulted in reduced weight gain. Growth response of fish fed diet 5 (containing 21.18 KJ/g, GE) sharply increased after 4 weeks, followed by diet 6, containing the highest dietary energy content (22.14 kJ/g, GE) (Figure 3.1). There was a trend of increasing growth performance with increasing inclusion level of dietary energy at each protein level on the basis of final body weight, percent weight gain and specific growth rate (SGR). This trend was not maintained above 21.18 kJ/g (43.52% protein and 8.23% lipid) diet 5 (P/E ratio 20.54 mg protein per kJ, GE), which produced best growth performance.

Diet 5 showed the lowest coefficient of variance (CV) of final weight, followed by diet 6 but no significant differences ($P > 0.05$) were found between treatments.

Food conversion efficiencies (FCE) increased as the dietary energy level increased at each protein level being highest ($P < 0.05$) for diet 5 and lowest for diet 1. This trend was not maintained above 21.18 kJ/g gross energy where FCE declined. No significant differences ($P > 0.05$) were found in FCE between the higher protein diets (Table 3.3).

3.3.2 Nutrient and Energy Utilisation

Protein utilisation efficiency, measured in term of protein efficiency ratio (PER) and apparent net protein utilisation (ANPU), is presented in Table 3.3. The protein efficiency ratio (PER) increased but not significantly ($P > 0.05$) as dietary protein level increased. Dietary energy increased up to 21.18 kJ/g of diet 5 beyond that showed decrease PER value. However, no significant differences ($P > 0.05$) were found between the treatments.

Apparent net protein utilisation (ANPU) was not significantly affected ($P > 0.05$) by increasing inclusion level of dietary protein and energy, similarly to PER (Table 3.3). The highest value was produced by diet 5 (43.51% protein and 21.18kJ/g gross energy, corresponding to P/E ratio 20.54 mg protein per kJ of GE). Since body protein contents (Table 3.4) of treatment groups were almost similar, ANPU values in general tended to reflect PER. The apparent net lipid utilisation (ANLU) tended to increase at lower energy (lipid) levels at each protein level ranging from 71.36% to 135.76%.

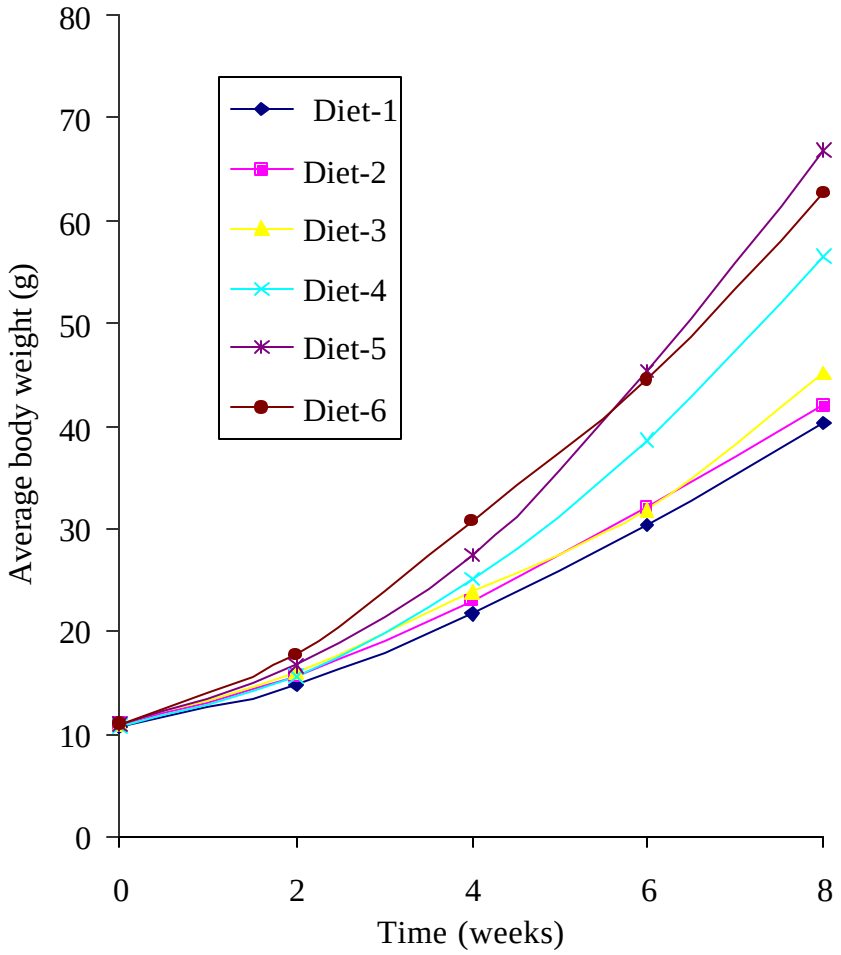

Figure 3.1 The mean fortnightly growth response of African catfish, *Clarias gariepinus* maintained on the six experimental diets over 8 weeks**.**

Diets 1, 2, 3, 4, 5 and 6 contained P / E ratio 17.09, 16.28, 15.49, 21.28, 20.54 and 19.55 mg protein per kJ of GE respectively. Fish were fed at 5% body weight day^{-1} at 28 ± 1°C.

Table 3.3 Mean growth performance, feed and nutrient efficiency of *Clarias gariepinus* fed various protein to energy ratios for 56 days

	Diet number (Designation: Protein / Lipid, %)						
Diet no. (Protein / Lipid, %)	1 (33/4)	2 (33/8)	3 (33/12)	4 (43/4)	5 (43/8)	6 (43/12)	± SEM
Parameters:							
Initial body wt. (g)	10.85[a] ± 0.11	10.93[a] ± 0.06	10.94[a] ± 0.18	10.85[a] ± 0.08	10.89[a] ± 0.11	10.90[a] ± 0.12	0.03
Final body wt. (g)	40.30[a] ± 10.34	42.07[a] ± 6.93	45.15[ab] ± 5.68	56.61[ab] ± 6.17	67.01[b] ± 8.24	62.72[b] ± 5.76	2.90
CV (%) of final body wt.	40.61 ± 2.65	40.71 ± 9.84	43.40 ± 4.36	43.81 ± 5.08	35.65 ± 4.85	36.25 ± 2.74	1.41
Weight gain (g)	29.45[a] ± 10.27	31.15[a] ± 6.87	34.21[a] ± 5.71	45.76[ab] ± 6.17	56.11[b] ± 8.35	51.81[b] ± 3.84	3.03
Percent wt. gain (%)	271.11[a] ± 93.51	284.84[a] ± 61.63	312.95[a] ± 52.82	421.71[ab] ± 56.50	515.68[b] ± 81.78	475.29[b] ± 30.13	27.90
Specific growth rate (SGR) (% day)	2.30[a] ± 0.50	2.39[ab] ± 0.30	2.52[ab] ± 0.24	2.94[ab] ± 0.19	3.24[b] ± 0.24	3.12[b] ± 0.17	0.11
Food conversion efficiency (FCE)	0.73[a] ± 0.13	0.74[a] ± 0.09	0.78[ab] ± 0.08	1.01[bc] ± 0.08	1.11[c] ± 0.04	0.98[bc] ± 0.09	0.04
Protein efficiency ratio (PER)	2.18[a] ± 0.39	2.11[a] ± 0.27	2.15[a] ± 0.25	2.29[a] ± 0.18	2.56[a] ± 0.09	2.28[a] ± 0.22	0.06
Apparent net protein utilisation (ANPU, %)	36.88[a] ± 9.48	36.74[a] ± 3.96	38.10[a] ± 4.95	41.74[a] ± 1.95	46.17[a] ± 0.49	37.52[a] ± 3.37	1.28
Apparent net lipid utilisation (ANLU, %)	127.80[b] ± 22.85	97.13[ab] ± 8.65	92.02[ab] ± 10.91	135.76[b] ± 27.12	110.19[ab] ± 8.77	71.36[a] ± 6.86	6.18
Apparent net energy utilisation (ANEU, %)	26.5[a] ± 5.82	28.63[a] ± 2.01	32.83[ab] ± 3.92	32.09[ab] ± 3.21	39.32[b] ± 1.02	32.69[ab] ± 2.90	1.21
Protein digestibility (%)*	89.83	89.50	89.36	91.95	93.02	92.52	
Lipid digestibility (%)*	72.67	84.81	73.75	71.98	90.12	89.08	
Energy digestibility (%)*	73.20	76.90	81.25	71.99	79.29	79.44	
Dry matter digestibility (%)*	70.18	73.74	78.87	68.64	75.52	76.11	

Note: Values are means ± SD of three replications (d. f. 5, 17). Means in the same row having different superscripts are significantly different ($P < 0.05$) and values in the same row with same superscript are not significantly different ($P > 0.05$).
* No statistical analysis was possible as determinations were performed on pooled samples.

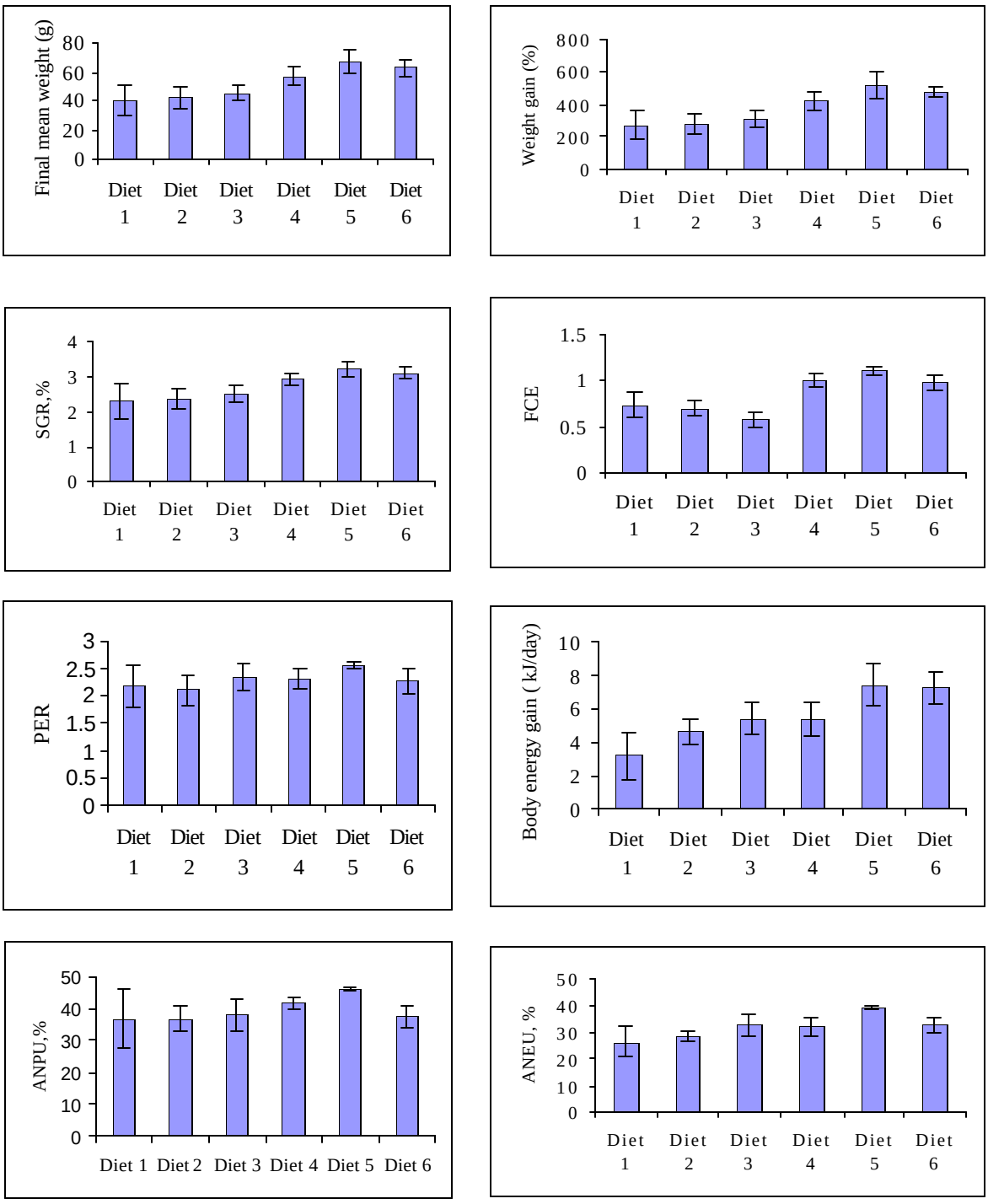

Figure 3.2 Mean growth performance, feed and nutrient efficiency of *Clarias gariepinus* fed various dietary protein to energy ratios for 56 days (SGR= Specific growth rate; FCE= Food conversion efficiency; PER= Protein efficiency ratio; ANPU= Apparent net protein utilisation; ANEU= Apparent net energy utilisation)

Bars are means ± SD of three replicates. Diets 1, 2, 3, 4, 5 and 6 contained dietary P/E ratios 17.09, 16.28, 15.49, 21.28, 20.58 and 19.55 in mg protein per kJ of GE respectively.

As shown in Table 3.3, apparent net energy utilisation (ANEU) was significantly ($P < 0.05$) influenced by the dietary energy level at each protein level. Fish fed diet 5 (P/E ratio 20.54) produced significantly ($P < 0.05$) the highest value and diet 1 the lowest. No significant differences ($P > 0.05$) were found between higher protein diets. Furthermore, increasing energy to 22.14 kJ/g resulted in no further improvement in nutrient and energy utilisation in the treatment groups.

3.3.3 Apparent Nutrient, Energy and Dry matter Digestibility

Apparent nutrient, energy and dry matter digestibility data are summarised in Table 3.3. Apparent protein digestibility values were fairly high ranging from 89.36% to 93.02%. Protein digestibility was slightly higher in the high protein diets and was not substantially affected by dietary energy or lipid level. However, the highest protein digestibility value (93.02%) was found in the diet 5, containing 21.18 kJ/g gross energy (corresponding to a P/GE ratio of 20.54 mg protein per kJ). The lowest protein digestibility (89.83%) was found in the diet 1 containing the lowest total energy. Protein digestibility values tended to increase as dietary energy increased up to 21.18 kJ/g (Table 3.3).

As shown in Table 3.3, apparent lipid digestibility was also fairly high and ranged from 71.98% (diet 4) to 90.12% (diet 5). Apparent energy digestibility ranged from 71.99% (diet 4) to 81.25% (diet 3). Dry matter digestibility values ranged from 70.18% (diet 1) to 78.87% (diet 3).

3.3.4 Body Composition and Histology

Body composition data for whole fish carcasses are shown in Table 3.4. At the end of the experiment, compared to initial values, all experimental groups exhibited higher percentages of protein and lipid, lower percentages of moisture and similar percentages of ash. In general, there was a trend towards higher body lipid contents and lower body moisture and protein content with increasing dietary energy level at each protein level, sometimes significantly. Higher protein level diets also resulted in significantly ($P < 0.05$) lower carcass lipid contents and insignificantly ($P > 0.05$) higher moisture and protein contents than the lower protein diets (Table 3.4).

Body protein content was highest in fish fed diet 5 and lowest in fish fed diet 3. Body lipid content was highest in fish fed diet 3 and lowest in fish fed diet 4. The trend was of increasing ($P < 0.05$) body lipid content with increasing dietary energy at each protein level and this increase was less noticeable at the higher protein level (Table 3.4). Body lipid content was positively correlated ($Y = - 8.6 + 0.83X$; $r = 0.16$; $P < 0.05$) with dietary energy content. Body moisture content decreased with increased dietary energy level and the highest value was found for the lowest dietary energy level at each protein level. There was an inverse relationship between moisture and lipid content. Body ash content ranged from 2.30% (diet 5) to 2.66% (diet 6).

As shown in Table 3.5, liver lipid level increased with dietary lipid level at each protein level and liver lipid levels were higher at the lower protein level. Deposition of liver lipid showed a strong correlation ($Y = -15.4 + 1.35X$; $r = 0.17$; $P < 0.05$) with dietary energy level.

Table 3.4 Body composition (% wet weight) of *Clarias gariepinus* at the start and end of the experiment

		Diet number (Designation: Protein / Lipid, %)						
Diet no.:	Initial	1	2	3	4	5	6	±
Parameters (%):		(33/4)	(33/8)	(33/12)	(43/4)	(43/8)	(43/12)	SEM
Moisture	74.12	73.28^{b} ± 1.44	70.63^{ab} ± 0.91	69.74^{a} ± 0.73	73.57^{b} ± 1.15	71.98^{ab} ± 0.99	70.93^{ab} ± 0.33	0.39
Crude Protein	15.52	16.45 ± 1.14	16.88 ± 0.78	16.05 ± 0.58	17.70 ± 0.79	17.74 ± 0.59	17.31 ± 0.42	0.21
Crude Lipid	7.74	7.67^{b} ± 0.04	10.00^{c} ± 0.85	11.43^{d} ± 0.15	6.14^{a} ± 0.67	8.07^{b} ± 0.62	9.10^{bc} ± 0.40	0.43
Ash	2.63	2.60 ± 0.28	2.51 ± 0.24	2.61 ± 0.11	2.59 ± 0.35	2.30 ± 0.14	2.66 ± 0.29	0.06

Note: Values are means ± SD of three replications. Means in the same row having different superscripts are significantly different ($P < 0.05$) and values in the same row with no superscript are not significantly different ($P > 0.05$).

Table 3.5 Liver lipid, liver glycogen (% wet weight), viscerosomatic index (VSI) and hepatosomatic index (HSI) of *Clarias gariepinus*

	Diet number (Designation: Protein / Lipid, %)						
Diet no.:	1	2	3	4	5	6	±
	(33/4)	(33/8)	(33/12)	(43/4)	(43/8)	(43/12)	SEM
Parameters (%):							
Liver Lipid	11.52^{ab} ± 3.18	14.58^{bc} ± 0.58	16.03^{c} ± 0.85	7.51^{a} ± 1.08	13.33^{bc} ± 1.21	13.63^{bc} ± 1.17	0.75
Liver Glycogen	1.03^{b} ± 0.07	0.84^{ab} ± 0.04	0.81^{ab} ± 0.06	0.89^{ab} ± 0.14	0.76^{a} ± 0.12	0.74^{a} ± 0.09	0.03
VSI	8.18 ± 1.26	8.23 ± 0.81	8.55 ± 1.44	6.47 ± 1.37	7.64 ± 1.83	7.89 ± 0.38	0.31
HSI	2.18 ± 0.24	2.22 ± 0.52	2.45 ± 0.33	1.61 ± 0.24	1.87 ± 0.42	1.71 ± 0.04	0.09
Liver glycogen (mg per fish)[1]	9.05	7.85	8.96	8.11	9.52	7.94	

Note: Values are means ± SD of three replications. Means in the same row having different superscripts are significantly different ($P < 0.05$) and values in the same row with no superscript are not significantly different ($P > 0.05$).

1. Calculated from mean fish weight and HSI data.

Liver glycogen content (Table 3.5) was highest ($P < 0.05$) fish fed diet 1, and lowest ($P < 0.05$) in fish fed diet 6. There was an overall trend of decreasing liver glycogen content with increasing dietary energy level at each protein level. Higher protein diets showed lower liver glycogen content. Liver glycogen was significantly ($P < 0.05$) negatively correlated with dietary energy ($Y = 3.10 - 0.108X$; $r = 0.87$; $P < 0.05$). VSI and HSI did not vary significantly ($P < 0.05$) among the test groups. There was a trend toward lower VSI and HSI in higher protein diets (Table 3.5).

If the relationship between HSI and fish weight is used to calculate the liver glycogen contents in mg per fish (Table 3.5) it can be seen that this is fairly constant for all treatments.

Results from the histological examination of intestine and liver found no significant abnormalities in response to experimental diets.

3.3.5 Digestive Enzymes

Protease and lipase activities in intestine and liver are presented in Table 3.6 and Figure 3.3. No consistent trends in protease and lipase activities with changes in dietary lipid or protein level were apparent. In general, protease and lipase activity was found to be greater in the intestine than in liver.

Table 3.6 Protease & lipase activities in *Clarias gariepinus* at the end of the experiment

	Diet number (Designation : Protein / Lipid, %)						
Diet no.:	1	2	3	4	5	6	±
(Protein / Lipid, %)	(33/4)	(33/8)	(33/12)	(43/4)	(43/8)	(43/12)	SEM
Enzymes:							
Intestinal protease[1]	83.54 ± 11.54	82.93 ± 13.64	78.74 ± 9.66	77.65 ± 4.97	87.18 ± 1.01	82.49 ± 1.80	1.86
Intestinal lipase (EC 3.1.1.3)[2]	1.60 ± 0.70	2.03 ± 0.81	2.17 ± 0.47	1.90 ± 0.36	1.97 ± 0.31	1.93 ± 0.32	0.11
Liver protease[1]	83.68 ± 16.15	79.18 ± 6.06	74.02 ± 5.13	73.95 ± 4.12	85.29 ± 12.93	81.35 ± 6.97	2.18
Liver lipase (EC 3.1.1.3)[2]	1.21 ± 0.10	1.47 ± 0.59	1.87 ± 0.70	1.70 ± 0.44	1.87 ± 0.21	1.67 ± 0.29	0.10

Note:

1. Protease activity was expressed as the amount of protein (µg) digested by 0.5 ml of enzyme solution at pH 7.6 per min. at 30°C.
2. Lipase activity was expressed as the amount of fatty acids (Sigma / Tiez unit/L) liberated by 1ml of extracted enzyme solution per min. at 30°C.

Values are means ± SD of three replications. Values in the same row are not significantly different ($P > 0.05$).

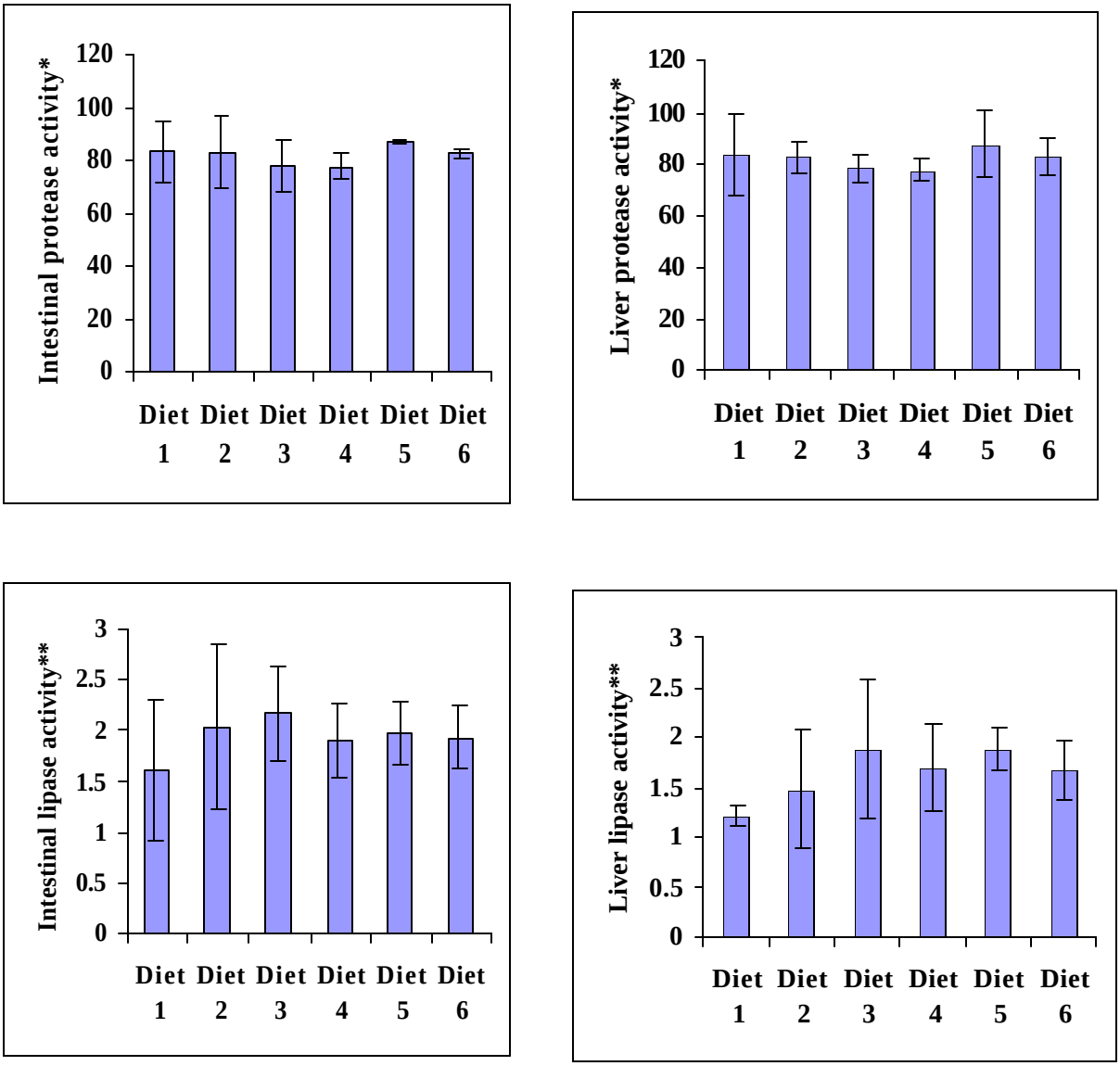

Figure 3.3 The protease and lipase activities of intestine and liver in *Clarias gariepinus* fed different experimental diets.

Bars are means ± SD of three replications. Diets 1, 2, 3, 4, 5, and 6 contained P/GE ratios 17.09, 16.28, 15.49, 21.28, 20.54 and 19.55 mg protein per kJ of GE respectively.

* Protease activity was expressed as the amount of protein (µg) digested by 0.5 ml of enzyme solution at pH 7.6 per min. at 30°C.

** Lipase activity was expressed as the amount of fatty acids (Sigma / Tiez unit/L) liberated by 1ml of extracted enzyme solution per min. at 30°C.

3.3.6 Blood Plasma Components

The concentrations of plasma components such as glucose, triglycerides (TG) and cholesterol are shown in Table 3.7 and Figure 3.4. Plasma glucose concentration tended to decrease significantly ($P < 0.05$) with increasing dietary energy level at each dietary protein level. Plasma glucose concentration was lowest ($P < 0.05$) in fish fed diet 6 (high protein, high lipid). The highest ($P < 0.05$) plasma glucose level was found in fish fed diet 1, containing low protein and low lipid.

As shown in Table 3.7, the plasma concentration of triglycerides (TG) and cholesterol increased significantly ($P < 0.05$) with increasing dietary energy (as lipid) in the lower protein diets. The highest TG level was found for the high lipid diets at both protein diets. The highest TG value was recorded for diet 3 (high lipid, low protein) which also exhibited the highest cholesterol concentration. Moreover, plasma TG and cholesterol levels were positively correlated with dietary energy (TG, $Y = -719 + 44.6X$, $r = 0.77$, $P < 0.05$; cholesterol, $Y = -1070 + 62.5X$, $r = 0.99$, $P < 0.05$), and significant differences were recorded in these concentrations between diets 1 and 3.

Blood plasma free amino acid levels in *Clarias gariepinus* are summarised in Table 3.8. Most of the free essential and non-essential amino acid levels in plasma were slightly ($P > 0.05$) higher in fish fed lower dietary protein levels. The both essential and non-essential amino acid levels in the plasma did not show any consistent trends with changes in dietary energy (as lipid) levels at the both protein levels.

Table 3.7 Blood plasma concentrations of glucose, triglycerides and cholesterol of *Clarias gariepinus* at the end of the experiment

	Diet number (Designation: Protein / Lipid, %)						
Diet no.: **Components:**	1 (33/4)	2 (33/8)	3 (33/12)	4 (43/4)	5 (43/8)	6 (43/12)	± SEM
Glucose (mg/100 ml)	66.34^{b} ± 7.13	64.77^{b} ± 7.63	41.23^{ab} ± 11.04	61.28^{b} ± 4.60	53.89^{ab} ± 13.44	33.11^{a} ± 8.25	3.54
Triglycerides (mg/100ml)	160.20^{a} ± 8.59	169.49^{a} ± 41.56	248.87^{b} ± 59.98	130.64^{a} ± 23.65	124.82^{a} ± 11.72	170.59^{a} ± 18.28	17.20
Cholesterol (mg/100 ml)	150.90^{a} ± 7.63	205.38^{ab} ± 23.67	271.92^{b} ± 66.37	199.06^{ab} ± 12.31	202.28^{ab} ± 23.02	182.64^{ab} ± 33.88	11.10

Note: Values are means ± SD of three replications. Means in the same row having different superscripts are significantly different ($P < 0.05$).

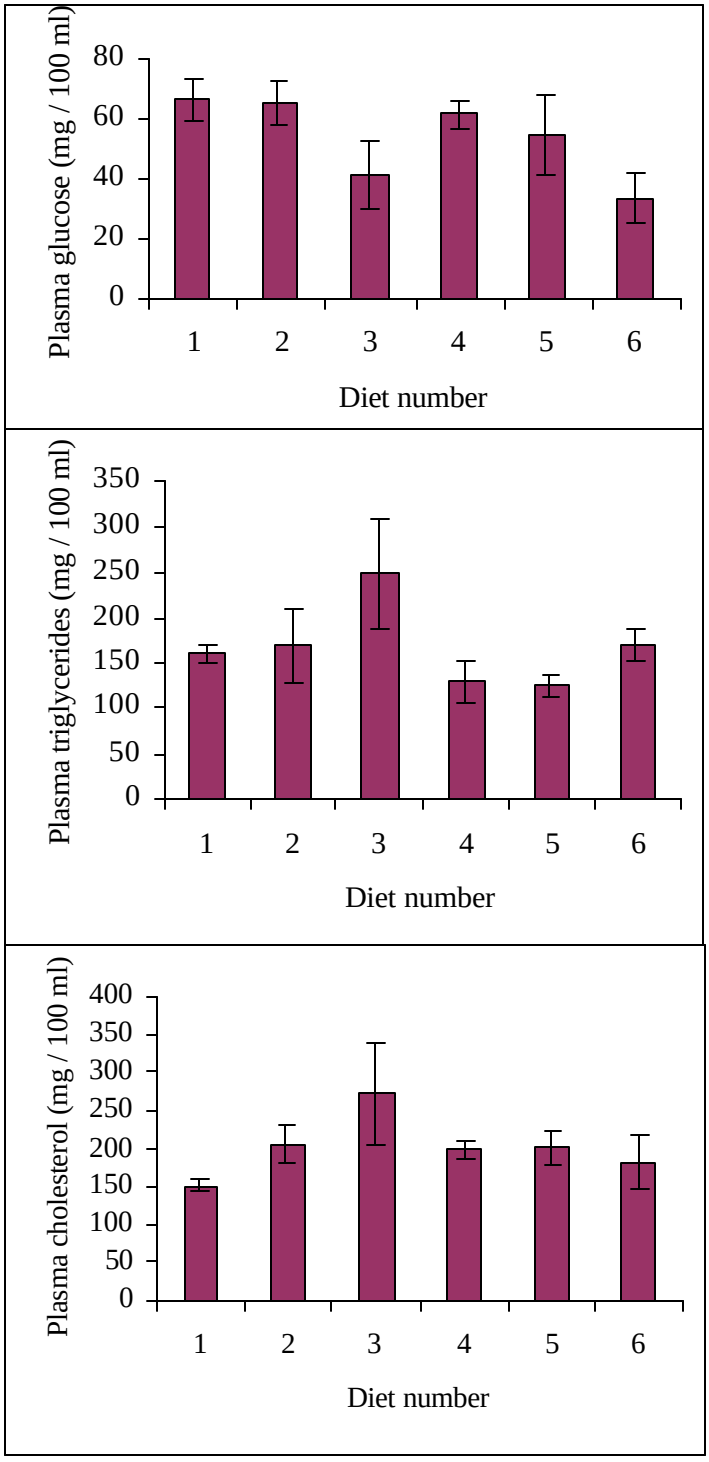

Figure 3.4 Blood plasma concentrations of glucose, triglycerides and cholesterol in *Clarias gariepinus* fed various dietary protein to energy ratios

Bars are mean ± SD of three replications. Diets 1, 2, 3, 4, 5 and 6 contained dietary P/GE ratios 17.09, 16.28, 15.49, 21.28, 20.54 and 19.55 mg protein per kJ of GE respectively.

Table 3.8 Plasma amino acid levels (μM / ml of blood plasma) in *Clarias gariepinus* at the end of the experiment.

	Diet Number (Designation : Protein / Lipid, %)						
Diet no.:	1	2	3	4	5	6	±SEM
(Protein / Lipid, %)	(33/4)	(33/8)	(33/12)	(43/4)	(43/8)	(43/12)	
Essential amino acids:							
Arginine	0.102 ± 0.006	0.110 ± 0.038	0.112 ± 0.065	0.078 ± 0.005	0.071 ± 0.007	0.067 ± 0.001	0.01
Histidine	0.074 ± 0.003	0.082 ± 0.013	0.075 ± 0.020	0.077 ± 0.007	0.060 ± 0.011	0.052 ± 0.005	0.00
Isoleucine	0.129 ± 0.025	0.088 ± 0.023	0.121 ± 0.014	0.098 ± 0.022	0.100 ± 0.005	0.109 ± 0.027	0.01
Leucine	0.226 ± 0.030	0.220 ± 0.039	0.242 ± 0.030	0.187 ± 0.048	0.203 ± 0.003	0.196 ± 0.031	0.01
Lysine	0.237 ± 0.016	0.258 ± 0.067	0.271 ± 0.107	0.215 ± 0.017	0.209 ± 0.024	0.189 ± 0.004	0.01
Methionine	0.042 ± 0.009	0.035 ± 0.014	0.033 ± 0.010	0.038 ± 0.012	0.038 ± 0.003	0.043 ± 0.023	0.00
Phenylalanine	0.077 ± 0.014	0.085 ± 0.020	0.067 ± 0.009	0.093 ± 0.019	0.066 ± 0.015	0.071 ± 0.022	0.00
Threonine	0.414*	0.445*	0.618*	0.386*	0.555*	0.552*	
Valine	0.201 ± 0.033	0.179 ± 0.026	0.213 ± 0.018	0.171 ± 0.028	0.193 ± 0.011	0.182 ± 0.023	0.01
Non-essential amino acids							
Alanine	0.385 ± 0.092	0.380 ± 0.071	0.450 ± 0.044	0.335 ± 0.103	0.330 ± 0.014	0.302 ± 0.035	0.02
Aspartic acid	0.050 ± 0.019	0.048 ± 0.019	0.035 ± 0.017	0.035 ± 0.012	0.030 ± 0.022	0.022 ± 0.10	0.00
Cystine	0.081 ± 0.030	0.084 ± 0.016	0.088 ± 0.031	0.084 ± 0.036	0.068 ± 0.018	0.068 ± 0.021	0.01
Glutamic acid	0.077 ± 0.021	0.083 ± 0.020	0.085 ± 0.025	0.060 ± 0.024	0.054 ± 0.018	0.041 ± 0.021	0.01
Glycine	0.227 ± 0.009	0.244 ± 0.097	0.213 ± 0.040	0.318 ± 0.097	0.263 ± 0.070	0.230 ± 0.054	0.02
Proline	0.251 ± 0.050	0.204 ± 0.085	0.150 ± 0.060	0.129 ± 0.099	0.114 ± 0.020	0.126 ± 0.012	0.01
Serine	0.295*	0.266*	0.308*	0.215*	0.297*	0.213*	
Tyrosine	0.069 ± 0.010	0.054 ± 0.014	0.053 ± 0.010	0.059 ± 0.024	0.064 ± 0.013	0.055 ± 0.032	0.00

Note: Tryptophan, an essential amino acid, not measured by the technique.

* No data in one/two replicates, as peak not differentiated in the chromatogram.

Values are means ± SD of three replications and values in the same row with no superscript are not significantly different ($P > 0.05$).

3.4 DISCUSSION

3.4.1 Growth and Feed Performance

At both dietary protein levels, poorest growth (% gain, SGR) was recorded for the lowest energy (as lipid) level diets. At low dietary energy levels protein may be used for energy, as has been demonstrated in *Clarias* catfish (Machiels and Henken, 1985; Jantrarotai *et al*., 1998) and other species (Cowey, 1980; El-Sayed and Teshima, 1992; Hassan *et al*., 1995; Yamamoto *et al*., 2000). Dietary energy may the primary source of variation in growth rate with lower weight gain for low energy diets a result of insufficient energy consumption. The fixed ration level (5% of body weight) used here might also have prevented the fish from consuming more feed to compensate for energy supply from low energy diets. As a result, fish presumably catabolised dietary protein to meet some of its requirements for energy rather than depositing it as growth. A gradual increase in growth with each incremental level of dietary energy up to 21.18 kJ/g GE strengthens the assumption that with increased energy more protein was utilised for tissue building and hence growth with supplementation above 21.18 kJ/g dietary gross energy (diet 5) growth rate declined.

Best growth was achieved at 43% protein, 8% lipid (diet 5) and 21.18 kJ/g gross energy. A P/E ratio of 20.54 (20.54 mg protein/ kJ of GE) or 33.43 (33.43 mg protein/ kJ of DE) is suggested for optimum growth, in agreement with earlier studies on this species (Machiels and Henken, 1985). However, increasing dietary lipid above 8% at the high protein level did not further improve fish growth in terms of percent weight gain or SGR. Similar trends have been obtained in *Clarias gariepinus* (Machiels and Henken, 1985), Tilapia (Winfree and Stickney,

1981; Teshima *et al.*, 1985a; El-Sayed, 1987; De Silva *et al*., 199; El-Sayed and Teshima, 1992) and Indian major carp (Hassan *et al.*, 1995; Hassan and Jafri, 1996).

The coefficient of variation (CV) of final body weights varied slightly in *Clarias gariepinus* during the present study. Diet 5 (P/E ratio 20.54 kJ/GE) had the lowest CV in weight and resulted in best growth performance and feed utilisation.

In the present study changes in food conversion efficiency (FCE) were generally very small although FCE was improved in the higher protein diets. At the lower protein level FCE improved very slightly with increasing lipid/energy level. At the high protein level FCE was best for the intermediate (8% lipid) diet (5). Improved feed conversion efficiency, up to a certain level of dietary energy inclusion (through lipid), has also been reported by earlier workers (Dupree *et al*., 1979; Shiau and Huang, 1990; De Silva *et al*., 1991; Hassan *et al.*, 1995; Jantrarotai *et al.*, 1998; Yamamoto *et al.*, 2000).

Clarias gariepinus fed the high protein, high lipid diet (6) exhibited a reduction in FCE. This could be attributed to reduced feed intake by those fish, because of the high energy content of the diet, resulting in lower protein intake (Page and Andrews, 1973; Grove *et al.*, 1978; Daniels and Robinson, 1986), or to the hindrance of digestion and absorption of other nutrients by the high energy content in the diet (Dupree *et al*., 1979).

3.4.2 Nutrient and Energy Utilisation

Protein utilisation, measured in terms of PER and ANPU, increased to some extent with increasing dietary lipid at both protein levels suggesting improved use of dietary protein for growth up to 21.18 kJ/g GE (diet 5). PER and ANPU declined for diet 6 containing the highest dietary energy level. The pattern of changes in PER and ANPU in relation to dietary energy is similar to observations on hybrid *Clarias* catfish (Jantrarotai *et al*., 1998), Indian major carp (Hassan *et al*., 1995), Nile tilapia (El-Sayed and Teshima, 1992) and African catfish, *Clarias gariepinus* (Machiels and Henken, 1985). Fish fed low protein and low energy diets showed poorer protein utilisation (ANPU) presumably because more protein was used for energy and a smaller amount for growth. Several factors, including dietary energy content and source, influence protein utilisation (Steffens, 1981).

In *Clarias gariepinus*, dietary protein and energy levels and their interaction seemed to influence energy conversion. In this study it was found that apparent lipid utilisation (ANLU) trended to increase at low lipid levels of each protein diets. The apparent energy utilisation (ANEU) increased with increasing energy level in the diets. At the higher dietary protein level, energy conversion reached a plateau beyond 21.18 kJ/g of GE (12.86 kJ/g of DE) incorporation in the diet. This assumption is in agreement with previous studies in *Clarias gariepinus* (Machiels and Henken, 1985), American eel (Tibbetts *et al.*, 2000) and Indian major carp (Hassan *et al.*, 1995).

3.4.3 Nutrients, Energy and Dry matter Digestibility

Apparent protein digestibility (APD) values were observed to be highest (93%) at the higher dietary protein level but were not affected by the inclusion level of dietary lipid. Values here are in agreement with those reported for *Clarias gariepinus* and other species by various authors (Machiels and Henken, 1985; Santinha *et al*., 1996; Appleford and Anderson, 1997). It was also found that an increase in lipid level had no effect on protein digestibility as demonstrated by other studies (Page and Andrews, 1973; Dela Higuerra *et al.*, 1977; Lorico-Querijero and Chiu, 1989).

At both protein levels the 8% lipid diet resulted in better apparent lipid digestibility than either 4% or 12%. This result is difficult to explain but may indicate that *Clarias gariepinus* is unable to secrete sufficient bile/lipase to meet digestive demands at the highest lipid level (12%).

At each protein level, apparent energy and dry matter digestibility increased slightly with increasing dietary lipid level in agreement with the trends reported earlier for *Clarias gariepinus* (Machiels and Henken, 1985) and gilthead sea bream (Santinha *et al*., 1996). However, energy and dry matter digestibility values increased as dietary fibre level decreased and it may be that fibre level was the principal factor involved here.

3.4.4 Body Composition and Histology

Carcass analysis indicated that carcass lipid increased and moisture decreased with increasing dietary lipid level at each protein level. Carcass lipid levels were higher in fish fed the lower protein diets. These observations seem in general agreement with results reported for African catfish, *Clarias gariepinus* (Machiels and Henken, 1985; Henken *et al.*, 1986), Indian major carp (Hassan *et al*., 1995), trout (Yamamoto *et al*., 2000) and *Colossoma macropomum* (Van der Meer *et al*., 1995, 1997). These results œuld also support the argument that a higher dietary protein level is not likely to cause a significant increase in body lipid content as conversion of amino acids to deposited lipids has an energetic efficiency of only 53% (Black, 1995). Fattiness is often undesirable in fish cultured for food and increasing the dietary protein level may be a strategy for producing a leaner product.

Body lipid and body moisture were inversely related while body protein and body ash contents remained almost constant as reported in previous experiments with African catfish, *Clarias gariepinus* (Hogendoorn, 1983b; Machiels and Henken, 1985; Henken *et al.*, 1986).

As with carcass lipid, increasing dietary lipid at each protein level also resulted in greater accumulation of liver lipid, with the lower protein diets having the higher liver lipid deposition. The positive correlation noted between dietary energy and liver lipid content compares favourably with other results obtained for this species *Clarias gariepinus* (Pantazis, 1999).

Liver glycogen content gradually decreased with increasing dietary lipid at each protein level and a negative correlation was noted between dietary energy level and liver glycogen deposition. There was a tendency for greater deposition of glycogen in fish fed the lower protein diets. The observation seems in agreement with results reported for sunshine bass (Keembiyehetty and Wilson, 1998), but differs from those for striped bass (Nematipour *et al*., 1992a), where gradually increasing dietary lipid and energy resulted in greater accumulation of liver glycogen with higher protein diets. Lower dietary protein contents were achieved by higher carbohydrate levels possibly resulting in increasing liver glycogen accumulation. It has been reported for several fish species that liver glycogen content generally increased with increasing dietary carbohydrate (Garling and Wilson, 1977; Hilton and Atkinson, 1982; Nematipour *et al.*, 1992a).

Viscerosomatic indices (VSI) increased slightly with increasing dietary lipid and energy level (decreasing P/E ratio) at both dietary protein levels. Higher VSIs were found for lower protein diets in agreement with earlier studies (Watanabe, 1982; Nematipour *et al*., 1992a; Gallagher, 1999). Hepatosomatic indices (HSI) were insignificantly higher in fish fed the lower protein diets and there was a trends of gradually increased HSI with increasing dietary lipid and energy, probably due to higher liver lipid and liver glycogen accumulation as a result of lower dietary protein to energy ratios (Brown *et al.,* 1992; Nematipour *et al.,* 1992a; Jantrarotai *et al.,* 1996, 1998; McGoogan and Gatlin III, 1999).

Histological examination of the intestine and liver indicated that diet changes did not produce parenchymal cell damage or lipid / glycogen deposition in cellular vacuoles. Refstie and Austreng (1981) fed rainbow trout with a diet containing 41.6% nitrogen-free extracts for 282 days with no apparent pathological signs, but this diet reduced growth and produced larger livers than diets lower in carbohydrates. Other authors reported on experiments in which very high levels of dietary lipid were fed to fish without any growth depression or pathological effects (Hepher, 1988).

3.4.5 Digestive Enzymes

Dietary protein level generally influences protease activity in fish (Kawai and Ikeda, 1972; Shcherbina *et al.*, 1976; Muhkopadhyay *et al.*, 1978; Knauer *et al.*, 1996; Gangadhara *et al.*, 1997) although not in the present study. The highest protease activity in liver and intestinal tissues was recorded for diet 5 (P/E ratio 20.54 mg protein per kJ of GE) containing 21.18 kJ/g of GE. Lipase activity in both intestine and liver was very slightly influenced by the dietary energy level. At the lower protein level lipase activity increased with increasing dietary lipid level although this was not evident for the higher protein diets. The highest lipase activity in liver and intestinal tissues was also recorded for diet 5 (P/E ratio 20.54 mg protein per kJ of GE) containing 21.18 kJ/g of GE.

Comparable results in published literature on protease and lipase activities of intestine and liver in African catfish, *Clarias gariepinus* are currently lacking. Furthermore, variations in digestive enzyme activities may be related to the structure of protein, energy metabolism and duration of retention of feed in the digestive tract which in turn depends on the crude fibre and

physical consistency of the diet (Venkatesh *et al*., 1986). This highlights the need for further studies of the relative contributions of fat, protein and carbohydrate to energy metabolism in this species.

3.4.6 Blood Plasma Components

Plasma glucose levels decreased with increasing dietary lipid at each protein level. Similar results have been reported for African catfish, *Clarias gariepinus* (Pantazis, 1999) and carp (Shimeno *et al*., 1995b). The stress involved in obtaining blood samples for glucose analysis may have resulted in up to a doubling of concentration (Fletcher, 1984). However, all samples were collected in the same way so that differences between treatments are still believed to be valid.

The concentration of plasma triglycerides and cholesterol were showed positively correlated with dietary energy as lipid at both protein diets, and they were highest in fish fed on the highest-lipid and lower- protein diets. Similar observations have been reported for carp (Shimeno *et al.,* 1995b) and tilapia (Shimeno *et al.,* 1993).

In general, plasma amino acid levels tended to increase as dietary protein level increased (Yokoyama *et al*., 1994; Yamamoto *et al*., 2000). In this study, most of the essential and non-essential amino acid levels in the plasma of the lower protein fed fish were slightly higher than for the higher protein diets. Similar phenomena were also observed with channel catfish (Wilson *et al*., 1985). The differing responses in free amino acid levels indicated by this study could, in part, be attributed to different experimental procedures especially timing of blood

samples with respect to the last meal. Both essential and non-essential amino acid levels in the plasma did not show any consistent differences in response to varying dietary energy (as lipid) at both protein levels. Similarly, Wilson *et al.*, (1985) who compared the serum free amino acid of channel catfish fed low or high energy (lipid) diets of varying protein levels, did not find marked differences in free amino acid levels.

It appears that the liver must exert a major control function in regulating the circulating levels of free amino acids after a meal, because the circulating levels of both the total free amino acids and total free essential amino acids were very similar, regardless of the level of protein fed in this experiment. This apparent regulatory effect of the liver would appear to function in ensuring that the extrahepatic tissues could make maximum utilisation of dietary amino acids for protein synthesis. The absence of any apparent effect of the protein to energy ratio of the test diets on circulating amino acids levels appears to indicate that adequate or excess energy was present in all of the test diets to facilitate almost similar amino acid utilisation (Wilson *et al.*, 1985).

In conclusion, on the basis of growth performance, feed efficiency, protein and energy utilisation, nutrient digestibility, body composition, digestive enzyme activity and plasma component metabolites, it may be stated that the diet 5, containing 43% and 21.18 kJ/g protein and gross energy respectively, performed best. This diet presumably contained the most appropriate P/E ratio 20.54 (20.54 mg protein/ kJ of GE) in African catfish, *Clarias gariepinus*.